Detecting NET using Methylation-based Biomarkers and the Novel IMPRESS Technology

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Background

- **The methylome is a promising source of biomarkers** as it enables to:
  - Differentiate **NET vs. non-NET** samples
  - Distinguish NET samples based on **tissue of origin**
- **Bisulfite conversion is the major limitation** of current methylation detection techniques, complicating translation towards liquid biopsies
- We developed the **IMPRESS** (Improved Methylation Profiling using Restriction Enzymes and smMIP Sequencing) technology, which is bisulfite-free, cost-effective and allows multiplex analysis

Aims

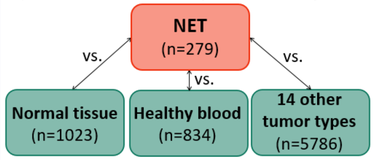
1) **Establish biomarker panels** of general NET and tissue-specific differentially methylated CpGs (DMCs)
2) **Validate our panels** in fresh frozen tissue from pancreatic, lung and small intestine NETs **using our IMPRESS technology**

Materials & Methods

Discovery

Biomarker panel 1:
NET or no-NET

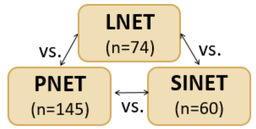
1) Differential Methylation analysis



2) Define general NET DMCs
3) Filter for IMPRESS application
   - Presence of restriction sites
   - Hypermethylated in NET

Biomarker panel 2:
Tissue-of-origin

1) Differential Methylation analysis



2) Define tissue-specific DMCs
3) Filter for IMPRESS application
   - Presence of restriction sites
   - Hypermethylated in specific NET type

Validation

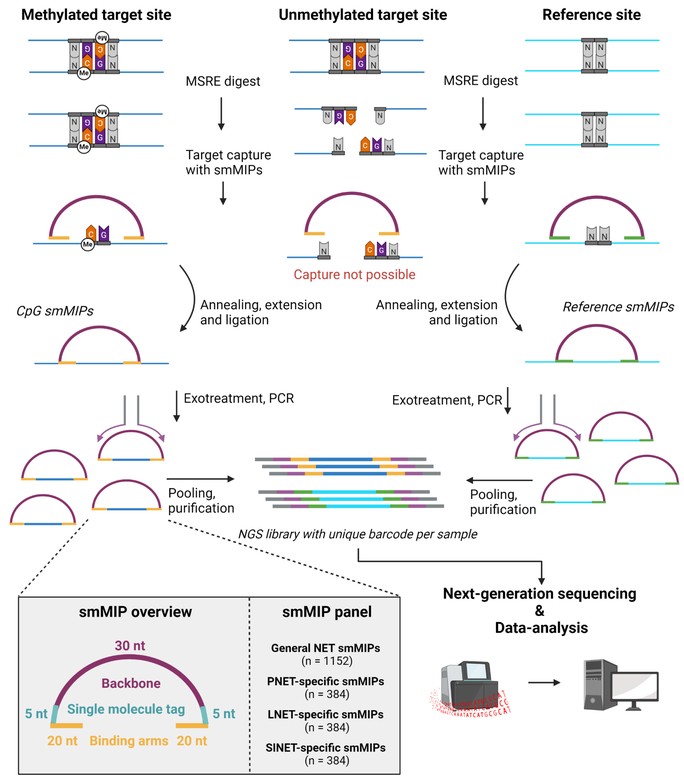


Figure 1 | Overview of the IMPRESS technology. *MSRE*; Methylation Sensitive Restriction Enzymes, *smMIP*; single-molecule Molecular Inversion Probe. Created with Biorender.com.

Table 1 | Overview of fresh frozen tissue samples used for validation.

Group	Sample type	Abbr.	# samples	Total
NET	Lung NET	PNET	2	6
	Pancreatic NET	SINET	2	
	Small intestine NET	LNET	2	
Healthy	Normal adjacent tissue	NT	6	10
	Healthy blood	HB	4	

Results

Normalized Counts **NET vs. Healthy**

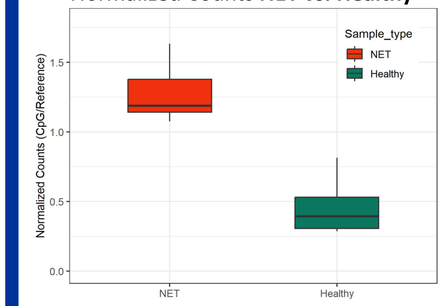


Figure 2 | This boxplot shows that NET (red, n = 5**) and healthy (green,** n = 8**) samples can be perfectly distinguished** based on the normalized counts generated by the smMIPs of the general NET panel. Normalization was performed by taking the ratio of the sum of the counts of the CpG smMIPs from the general NET panel and the sum of the counts of the reference smMIPs. One NET sample and two healthy samples did not pass quality control.

Normalized Counts **LNET vs. PNET vs. SINET**

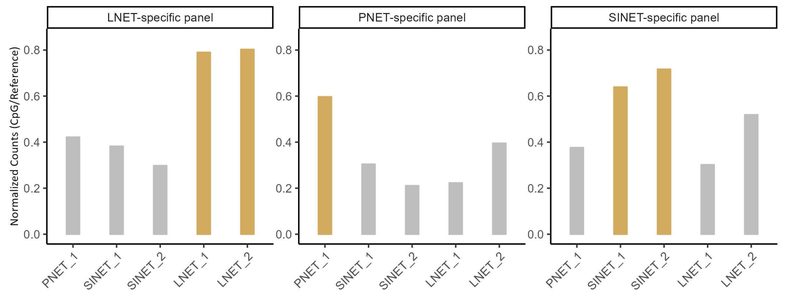


Figure 3 | This barplot indicates that NET samples can be distinguished based on tissue of origin using the normalized counts generated by tissue-specific smMIPs. Normalization was performed by taking the ratio of the sum of the counts CpG smMIPs from the tissue-specific panels and the sum of the counts of reference smMIPs. Normalized counts generated by LNET-specific smMIPs, were highest for LNET samples. The same applied for PNET- and SINET-specific smMIPs and the respective PNET and SINET samples.

Conclusions & Future Perspectives

- **We established** a general NET and tissue-specific **biomarker panels**.
- **Performed initial validation** and proved that we can **(i)** differentiate NET from healthy samples and **(ii)** differentiate NET samples based on tissue of origin using our biomarker panels and IMPRESS.
- More **extensive validation is ongoing**.